Review

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Skin Irritation and Sensitization: Mechanisms and New Approaches for Risk Assessment

1. Skin Irritation

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Abstract
Cutaneous irritation presents a major health problem with serious social and occupational impact. The interaction between an irritant and the human skin depends on multiple factors: the intrinsic properties and the nature of the irritant itself, and specific individual- and environment-related variables. The main pathological mechanisms of irritancy include skin barrier disruption, induction of a cytokine cascade and involvement of the oxidative stress network; all of them resulting in a visible or subclinical inflammatory reaction. In vivo, different non-invasive parameters for the evaluation of skin irritation and irritant potential of compounds and their specific formulations have been introduced, such as epidermal barrier function, skin hydration, surface pH, lipid composition, skin colour and skin blood flow. The diverse physiological changes caused by irritating agents require implementation of a multiparametric approach in the evaluation of cutaneous irritancy. Copyright © 2008 S. Karger AG, Basel

Introduction

Cutaneous irritation is the biological response of the skin to a variety of external stimuli that induce skin inflammation. Previously thought to be a monomorphic process, skin irritation is now considered a complex biologic event, with a distinct pathophysiology, natural history and clinical appearance. The host reaction to the offending exogenous agents embraces different clinical entities and depends on various factors of the irritating stimuli, environment and the host organism itself. A recent classification divides irritation based on the morphological and clinical course of the inflammatory reaction into 10 idiotypes [1].

Irritant contact dermatitis (ICD), defined as inflammatory response of the skin to an exogenous agent without requirement of prior sensitization, presents a major health problem with serious social and occupational impact [2, 3]. It is widely accepted that contact dermatitis compromises the vast majority of all skin diseases related to work factors [4]. The exposure to irritating agents at the workplace is a significant risk factor for developing occupational contact dermatitis (OCD) [2]. Patients with OCD are reported to have impaired quality of life similar to that of patients with acne, non-pressure urticaria, Darier's disease, Hailey-Hailey disease and Behçet's syn-

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drome [5]. In a study on 3,000 patients with registered occupational skin diseases, annual incidence rates of ICD were above 5.0 per 10,000 workers in hairdressers and barbers, employees in the food services, tile setters and terrazzo workers, florists, and metal workers [6]. In general, ICD was diagnosed slightly more often than allergic contact dermatitis [6]. The authors could not show a gender influence on the susceptibility to irritation in an occupational setting, in contrast to the results from former studies with a female predominance of irritant OCD [7].

Despite lacking the clinical signs of inflammation (redness, swelling, heat, pain and dysfunction) classically described by Rudolf Virchow [8], sensory or subjective irritation is a separate entity in the clinical spectra of irritation. Sensory irritation is characterized by developing itching, stinging, burning or tingling sensations on contact with certain substances, although the physical examination reveals no objective clinical signs [9].

The intrinsic properties of the irritants and their concentration play an important role in their interactions with the human skin [10]. Furthermore, the penetration of potentially irritant compounds or complexes depends on their net charge, molecular size as well as their molarity and their partition coefficient (log P) [11]. Comprehensive and systematic reviews of the nature and the biological effects of different irritants have been published [12, 13]. However, there is a lack of a unifying classification of irritating substances. Even in the *Globally Harmonized System of Classification and Labelling of Chemicals,* there are controversies about classifying the health hazards of irritant agents [14]. This concerns category 3 (mild irritants), which is only accepted by some authorities. The diversity of the nature and biological actions of the numerous irritating agents has led to the introduction of the REACH (Registration, Evaluation and Authorization of Chemicals) program in the EU. According to this new program, substances manufactured in or imported into the EU in volumes greater than 1,000 kg per year would have to be registered in a central database managed by a new European Chemicals Agency [15]. The regulation also calls for the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified.

Pathogenetic and Molecular Aspects of Irritation

The development of novel and sophisticated scientific methods elucidated some of the pathologic mechanisms in skin irritancy [16–19]. Previously described as an immunologic inert process, at present there is evidence that the process of irritation involves different cytokines and intercellular interactions [18–23]. The cytokine signal response is dependent on the dose and nature of the irritant itself [18, 19]. Interleukin-1α (IL-1α) plays a key role in the development of cutaneous irritancy [19, 23, 24]. It has been hypothesized that IL-1α is derived from the damaged keratinocytes during the interaction between the irritant and the epidermal barrier [19, 23]. Acute disruption of the epidermal barrier results in release of a preformed pool of cytokines, e.g. IL-1α, thus leading to an immediate initial irritant reaction and subsequently the induction of barrier repair mechanisms [23, 24]. Involvement of these immune mediators and their effects contributes to the clinical implementation of the irritant-induced inflammation of the skin. In repeated exposure to sodium lauryl sulphate (SLS), an elevated IL-1 receptor antagonist (IL-1 RA)/IL-1α ratio was observed, primary to an increase in IL-1α RA [20]. The balance between IL-1 RA and IL-1α has an important role in the regulation of the inflammatory response. In contrast, a decrease in the IL-1 RA/IL-1α ratio was previously demonstrated 24 h after an acute SLS patch test [22]. One may assume that this inconsistency is due to the dynamics of interleukin secretion in severe and chronically provoked irritancy. Furthermore, these immunological findings correspond to the slow decrease in the transepidermal water loss (TEWL) [25] and the epidermal hyperplasia [26] after repeated irritant exposure in experimental models of cumulative irritation. These results support the conception of the development of a hardening effect in the course of chronic irritancy. Regarding the epidermal barrier function it is hypothesized that upregulation of ceramide 1 synthesis plays a major role in inducing the hardening phenomenon in cutaneous irritation [27].

Interleukins play an essential role in the production of other cytokines and chemokines involved in inflammatory reactions. Irritation on human skin equivalent revealed IL-1α, together with tumour necrosis factor-α as primary signals for the release of secondary CCL20 and CXCL8 chemokine signals [19]. The pro-inflammatory effect of CCL20 and CXCL8 is performed by attracting a variety of immune cells, e.g. T cells, B cells, neutrophils and immature dendritic cells, and thus mediating the action of the epidermal residential cells in irritation [19].

The chemokine CCL21 was upregulated in response to provocation with 8 different chemical and 3 physical irritating stimuli [21]. CCL21 has been reported as the major mediator for dendritic cell migration from peripheral tissue [28]. The upregulation of CCL21 in cutaneous ir-

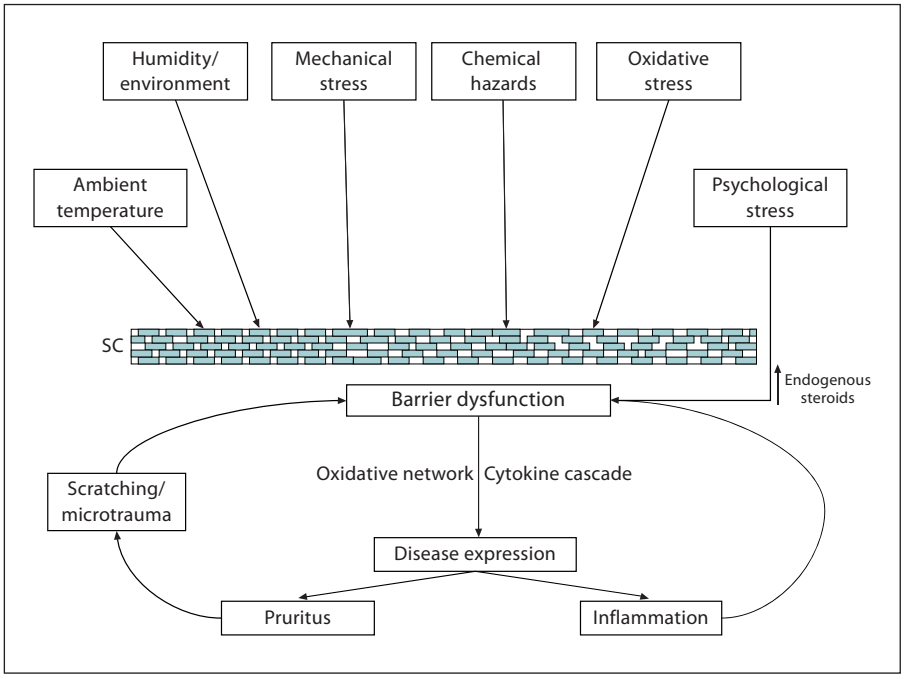

Fig. 1. Skin irritation and barrier function: influencing factors.

ritation may explain the previously described migration of Langerhans cells caused by irritant stimuli as a secondary phenomenon [21].

In vivo studies demonstrated increased levels of non-haematic iron, diene conjugates (as a function of lipid peroxidation) and oxidized glutathione in non-lesional skin of patients with chronic ICD and allergic contact dermatitis [29]. The elevated values of these parameters were previously linked to the activity of the oxidative stress network in skin inflammation in general [30–33]. Contact dermatitis, regardless its genesis, induces oxidative stress and its consequences.

Irritation and Skin Barrier Integrity and Function

The integrity of the epidermal barrier plays a pivotal role in the interaction and the response of human skin to irritating stimuli. The importance of an intact structure of the stratum corneum (SC) for susceptibility to irritants is well demonstrated in atopic dermatitis [34]. Patients with atopic skin diathesis demonstrate augmented response to a number of exogenous irritating agents corresponding to the impairment of the SC barrier in the disease [35, 36]. The importance of an intact epidermal barrier has been demonstrated by the detection of a mutation of the gene encoding filaggrin [37]. Filaggrin, as an essential part of the cornified envelope, plays a key role in maintaining the SC barrier homeostasis [38].

Nevertheless, not all irritants had disintegration of the skin barrier as an initial step in the development of irritation [39]. In dithranol-induced irritation, for example, barrier function and SC hydration are not initially altered [39, 40]. In support of these results, an in vivo study demonstrated a lack of upregulation of epidermal fatty acid-binding protein in response to irritation caused by dithranol [41]. Previously published data revealed upregulation of epidermal fatty acid-binding protein in the process of restoration of the SC barrier in SLS-induced irritation [42]. With regard to clinical implications, diverse irritation patterns require different measuring methods and evaluation of specific biologic endpoints in order to be discriminated [39].

Special practical interest is focused on the detergents and their interaction with the SC barrier in the context of the irritation process. Discrimination between surfactants belonging to different groups was observed by means of different non-invasive biophysical methods in a 48-hour single patch test exposure [43]. The results revealed SLS, followed by other anionic surfactants, as the detergent with the highest irritant potential. However, the exposure model and the evaluation methods are important variables influencing the outcome in irritancy testing [43, 44].

Combining detergents and physical irritants creates an additive effect on SC barrier impairment [45, 46]. Tandem repeated irritation tests (TRITs) demonstrated that combined mechanical irritation, SLS and occlusion cause complementary effects on the epidermal barrier disruption [45]. Further studies demonstrated the additive effect of chemical irritants of different nature, such as SLS, solvents and biogenic amines and fruity acids [47–49]. Application of such TRITs in irritant testing resembles to a greater extent the real conditions in everyday life and occupational settings than single-irritant exposure models.

Different mechanisms of barrier impairment are involved in various irritating stimuli. Organic solvents, e.g. acetone and toluol, extract the SC lipids, thus disrupting the entity of the epidermal barrier [50]. On the other hand, the anionic surfactant SLS damages protein structures such as keratin, which exposes new water-binding sites and causes hyperhydration of the SC and disorganization of the lipid bilayers [50, 51]. Regardless the nature of the affecting agent, acute barrier disruption induces processes of restoration of the homeostasis [52]. The triggering factor in the repair process is the increased water release through the impaired SC and the consequent reduction of calcium concentration in the stratum granulosum, thus inducing lamellar body secretion and lipid restoration [52]. A recent study revealed the interrelationship between nitric oxide levels, calcium concentration and barrier recovery [53].

Different factors modify the interactions between irritants and skin barrier. Figure 1 presents the interactions between exogenous stimuli and the epidermal barrier in skin irritation.

Methods and Models for Evaluation of Irritancy

Striving for quantification and qualification dates back to the establishment of ancient societies. Early Babylonian and Egyptian records and the Bible indicate that length was first measured with the forearm, hand, or finger [54]. The Vitruvian Man of Leonardo da Vinci is an example of the pioneer efforts to standardize certain measurement units using different body part ratios. Nowadays the demand for standardizing and unifying evaluation procedures in medicine is growing. Testing irritants was first performed on animals, e.g. Draize rabbit skin test [55, 56]. Recent ethical considerations resulted in the prohibition of animal testing with the 7th Amendment to the European Cosmetic Directive (76/768/EEC). The development of molecular investigative techniques led to the introduction of different in vitro models for the assessment of irritant properties [57]. Several comprehensive works give an overview of the in vitro and ex vivo models in irritancy testing [57–59]. At the time of this publication, the only in vitro models validated for predicting irritant properties by the European Centre for the Validation of Alternative Methods were EPISKIN® and EpiDerm® [60]. EpiDerm reliably identifies skin irritants, but negative results may require further testing [60]. Nevertheless, none of the existing in vitro models is capable of exactly reproducing the response of human skin to various irritating stimuli.

Investigation of irritancy in vivo implicates different models in terms of targeted aims, i.e. predicting susceptibility to irritants (susceptibility evaluation), exploring reaction patterns to different irritants, or studying the mechanism of the irritant reaction itself (provocative tests) [61]. Despite the application of defined visual score scales [61], an irritant reaction does not always include clinical signs, especially in the case of mild or subliminal irritation [62]. Subliminal irritation and the need for objectivity led to the introduction of various biophysical parameters such as TEWL, skin hydration, superficial pH, skin colour and skin blood flow [63]. Table 1 presents a summary of selected parameters for the in vivo assessment of skin irritation and individual- and environment-related variables influencing the measurements.

TEWL as a Measure of Epidermal Barrier Function

TEWL is one of the most important parameters to evaluate the epidermal permeability barrier function of the skin. A low TEWL is generally a characteristic feature of an intact skin function [64]. Thus, irritating agents compromising the skin barrier increase transepidermal evaporation. A study proved the validity of TEWL as a parameter reflecting the permeability barrier status by correlating ex vivo gravimetric measurements to absolute rates of water loss and determination of TEWL [64]. It was also disclosed that both open- and closed-chamber systems for TEWL quantification detect minor perturbations of human SC in vivo. However, certain limitations among different instruments have been revealed [64].

Furthermore, not only interinstrumental, but also interracial, interindividual, site, and different environmental variables affect TEWL measurements [65, 66]. The efforts to standardize and gain reproducibility led to the development of guidelines for TEWL measurements [66, 67].

Table 1. Influence of individual- and environment-related variables on biophysical skin measurements

a Individual-related variables

Parameter	Individual-related variables					
	age	gender	race	anatomical site	sweating	skin temperature
TEWL	+	∅	+/–	+	+	+
SC hydration	+	∅	n.d.	+	+	+
pH	+	+/–	+	+	+	+/–
Colour/erythema	+	+	+	+	n.d.	+
Microcirculation	+	+/–	∅	+	n.d.	+

b Environment-related variables

Parameter	Environment-related variables					
	air convection	ambient temperature	humidity	season	circadian rhythms	direct light
TEWL	+	+	+	+	+	+
SC hydration	+	+	+	+	+/–	n.d.
pH	n.d.	n.d.	n.d.	+	+	n.d.
Colour/erythema	∅	+	∅	+	+	+
Microcirculation	+	+	+	n.d.	+/–	+

+ = Influencing; ∅ = no influence; n.d. = no data; +/– = controversial data.

The anionic surfactant SLS is widely used in irritation investigations. It causes epidermal changes including: direct alteration of the lamellar body secretory system of the stratum granulosum cells; disturbance of the processing of lamellar body-derived sheets into bilayer structures in the intercellular spaces of the SC, and indirect induction of disturbed differentiation by altering the viable epidermis [68]. Many studies demonstrated that the course of the SLS-induced reaction evaluated by TEWL dynamics is dependent on different variables, e.g. concentration and time of exposure, site of exposure, microclimate changes, and the use of additive irritant stimuli [45–48, 61, 69].

In terms of irritant susceptibility testing one may assume that basal TEWL could be used as an appropriate parameter. Indeed, a number of results revealed a good correlation between pre- and post-exposure TEWL in SLS-induced irritation models, mainly after a single exposure [70–72]. Other investigations could not observe this correlation [73, 74], probably due to differences in the applied models and possible adaptation of the skin to multiple repeated exposures to SLS. However, one should keep in mind that these results are related to SLS-induced irritation and might not be valid for the susceptibility to irritants acting in a different manner than SC barrier disruption [39]. A multiparametric study proved that TEWL measurement is the most appropriate non-invasive modality to distinguish skin changes over time in experimental irritation induced by SLS and tape stripping, compared to irritation models with dithranol, UV irritation and tretinoin [39]. Moreover, TEWL changes showed superiority to laser Doppler velocimetry (LDV) in evaluating skin reactions to SLS in concentrations below 0.5%, at any application time in a single SLS exposure model [63].

Determining TEWL is useful in studying subjective irritation [9, 75, 76]. Trends toward higher baseline TEWL values measured on the nasolabial fold and cheek were reported in stingers compared to non-stingers.

SC Hydration

Water content of the SC affects barrier permeability and flexibility and also regulates the activity of hydrolytic enzymes involved in the process of normal corneocyte desquamation [77, 78]. Failure of the SC to retain water induces dryness, impairs barrier function and thus increases the susceptibility to irritants and facilitates development of contact dermatitis [79, 80]. Hydration of the

SC, which is exposed to the relatively dry environment, is maintained by the natural moisturizing factor [81, 82]. The natural moisturizing factor is produced in the lower part of the SC partially by enzymatic degradation of the protein filaggrin and is a highly hygroscopic and water-soluble mixture of amino acids, specific salts and other derivates [82].

Apart from the TEWL measurement, there is no generally accepted method for direct in vivo determination of SC hydration. Many techniques have been described: microwave, thermal, and spectroscopic, including nuclear magnetic resonance spectroscopy, infrared and Raman spectroscopy [82, 83]. However, the most commonly applied methods in use are measuring the electrical conductance, capacitance, or impedance as an indirect indication for SC hydration. Individual-, instrumental- and environment-related variables influence the electrical measuring methods [84].

Skin moisture and TEWL values are not always correlated in the course of the irritant reaction [39, 85]. In general, the tenside SLS possesses an exsiccation effect on the SC [45]. However, after initial exsiccation 1 h after patch removal hyperhydration was observed by capacitance measurement on day 1 after a 24-hour single SLS exposure [69]. The original skin condition re-emerged after 7 days. The authors attributed the increase in SC hydration to the damage of corneocytes. These results are consistent with the findings of an initial increase in the skin capacitance after SLS exposure; however, no hyperhydration was observed for other surfactants than SLS [43]. Corneosurfametry in vitro and ex vivo studies support the hypothesis of early SC damage by showing changes in the corneocytes induced by application of surfactants [86, 87].

Another example of the discrepancy between TEWL values and SC hydration is in the region of palmoplantar skin. Basal TEWL on the palm is higher than in any other skin region [88]. However, SLS application induces a decrease in TEWL on palmar skin [88]. One explanation of this phenomenon is the occlusion of the intracorneal eccrine sweat gland ducts due to structural changes in the thick SC by SLS [89]. Therefore, one cannot accept only TEWL as a parameter reflecting epidermal barrier function, but should also include SC hydration measurement, especially in occupational dermatology.

Tape stripping as a model of acute mechanical irritation also elevates SC hydration explained by disturbing the skin barrier and thus exposing the viable, water-rich epidermis to direct contact with the environment [39]. However, tandem application of mechanical irritation and SLS delayed the decrease in SC dehydration [48]. This was attributed to the wash-out effect of the potentially dehydrating SLS.

Beyond surfactants, skin capacitance measurement failed to distinguish skin changes over time in irritation induced by other model irritants, i.e. dithranol, UV and tretinoin [39].

A recently introduced microsensor technology, Skin Chip®, is useful in providing a detailed non-optical capacitance map of the skin surface in vivo [90]. Furthermore, the data revealed the effect of surfactants on the SC, i.e. initial hyperhydration followed by exsiccation [90].

One should keep in mind that the use of classical electrical methods does not give the actual gradient distribution of water in the SC. Novel techniques, e.g. confocal Raman microspectroscopy, are helpful in the determination of the water gradient over the whole extent of the SC [77, 91]. The method is based on the inelastic light scattering of different molecules. The proportion of the intensities of the Raman bands at certain shifts can be used to determine the water-to-protein ratio. Following mathematical equations, the water content is expressed in grams per 100 g of wet tissue [91].

Skin pH

The 'acid mantle' of the skin surface has long been known to play a key role in preventing infection [92]. Recent data demonstrated the role of pH in the regulation of epidermal permeability homeostasis and SC integrity/cohesion [93]. Despite a number of sophisticated techniques for the evaluation of SC acidity, e.g. pH-sensitive fluorescent dyes, fluorescent lifetime imaging [94], flat glass electrode measurements remain the most commonly used being simple, quick and reproducible. Furthermore, flat glass electrode assessment correlates well with the fluorescent dye methods [95]. Various endogenous (race, anatomical site, gender, age, circadian rhythm and concomitant disorder) and exogenous (e.g. seasonal variables, washing, use of cosmetics) factors affect the surface acidity [96] and should be considered in pH measurements. The incomplete development of the acidic mantle in neonates is an important element in the susceptibility to diaper dermatitis [97].

SC acidity is essential for epidermal barrier recovery, the latter being delayed at a neutral pH, due to disturbance in processing secreted extracellular SC lipids, while lipid secretion remains unimpaired. An acidic pH is critical for barrier homeostasis, in part because two key lipid-processing enzymes, β-glucocerebrosidase and acidic

sphingomyelinase, which generate a family of ceramides from glucosylceramide and sphingomyelin precursors, respectively [98], exhibit low pH optima [93]. Furthermore, pH is suggested to influence the formation of lipid bilamellar layers in the SC [99]. It was demonstrated that elevation of SC pH delays barrier recovery after acute disruption by tape stripping due to downregulation of at least one lipid-processing enzyme (β-glucocerebrosidase), thus impairing the formation of the lipid components of the SC [100]. Moreover, elevation of pH values produces abnormalities in SC integrity and cohesion due to activation of serine proteases with neutral pH optima, followed by degradation of desmoglein 1 and reduction of the corneodesmosome density [100]. Inhibition and/or blockade of secretory phospholipase A_2 and non-energy-dependent sodium-proton exchanger (both responsible for sustaining the acidic mantle) perturbs permeability homeostasis and SC integrity [101].

Although exposure of intact skin to neutral pH does not affect the basal epidermal barrier permeability in short-term experimental settings [93], sustained repetitive elevation in SC pH leads to an increase in the basal TEWL levels, without affecting SC hydration [100]. The latter could resemble an occupational, prolonged contact with alkaline substances. Concerning the increased SC pH and the impaired barrier function in alkaline detergent-induced dermatoses, the authors suggest that sustained pH increase may play a key role in worsening and/or therapeutic resistance of occupational skin diseases.

Skin Colour

Erythema, or skin redness, is a major feature of inflammatory reactions produced by the contact with irritating substances. Classically, erythema has been assessed subjectively by means of different clinical scales [102]. The perception of skin colour and redness due to inflammation is highly subjective and may be influenced by desquamation, pigmentation, and biochromophores [103]. The need for reliability, reproducibility and quantification has led to the development of different devices providing objective evaluation of skin colour. Distinct concepts are used in the different devices: (1) tristimulus reflectance colorimetric analysis of light reflected from skin structures, e.g. Chromameter (Minolta, Osaka, Japan), expressing the results using the CIE (Commission Internationale de l'Eclairage) 3-dimensional space values; (2) reflectance spectrophotometry, using either broadband or selected wavelengths in the visible range with measurement of absorbance and reflectance, and (3) skin colour image analysis [102, 103]. Narrowband reflectance spectrophotometry-based devices use quantification of selected light wavelengths corresponding to the colour of selected biochromophores (melanin, haemoglobin), e.g. Mexameter (C&K Electronic, Cologne, Germany). The results are presented by erythema and melanin indices.

Different variables could influence the results [102, 103]. Despite the interinstrumental variables, Clarys et al. [104] demonstrated moderately to highly significant linear correlations between CIE colour parameters and the erythema/melanin indices. However, the relationship between a* values and the erythema index is complex and also depends on skin pigmentation [104].

Assessing erythema by bioengineering methods is widely used in studying skin response to irritant stimuli [48, 69, 105]. Skin redness evaluated by increase in a* values was demonstrated in short- and long-term exposures to SLS [69, 105]. Higher SLS concentrations resulted in an earlier development of erythema and a* values reached higher peaks [105]. Furthermore, it was demonstrated that re-exposure to the surfactant gained faster and more intense reply [105]. Erythema persisted for 3–7 days on the SLS-treated sites in the single application model [69]. No statistically significant discrimination in time course reaction in a single SLS exposure model was detectable by tristimulus colourimetry and narrowband spectrophotometry [39]. However, the Chromameter is found useful in discriminating the response over time to dithranol and tape stripping, although the Mexamether was more effective in tretinoin- and UV-induced irritation [39]. These discrepancies were attributed to the different mechanisms of action of the tested irritants.

With regard to detection of early response to irritating stimuli, chromametry was not able to detect differences in the erythema response before it became visible, unlike LDV [62].

In a TRIT model, chromametry failed to register early erythematous changes while simulating mild irritation [45]. However, single application of 0.5% SLS followed by 5 min of mechanical irritation led to a pronounced increase in the a* values [45].

Topical application of corticosteroids, conventionally used for ICD therapy, induces blanching of the skin [102]. This effect is based on the changes of underlying microcirculation of the skin. Usually the intensity of blanching after topical application of corticosteroids is evaluated subjectively by a trained observer using a visual score. In order to obtain objectivity, non-invasive instrumental evaluation of the skin colour changes is applied in determining the blanching effect [102].

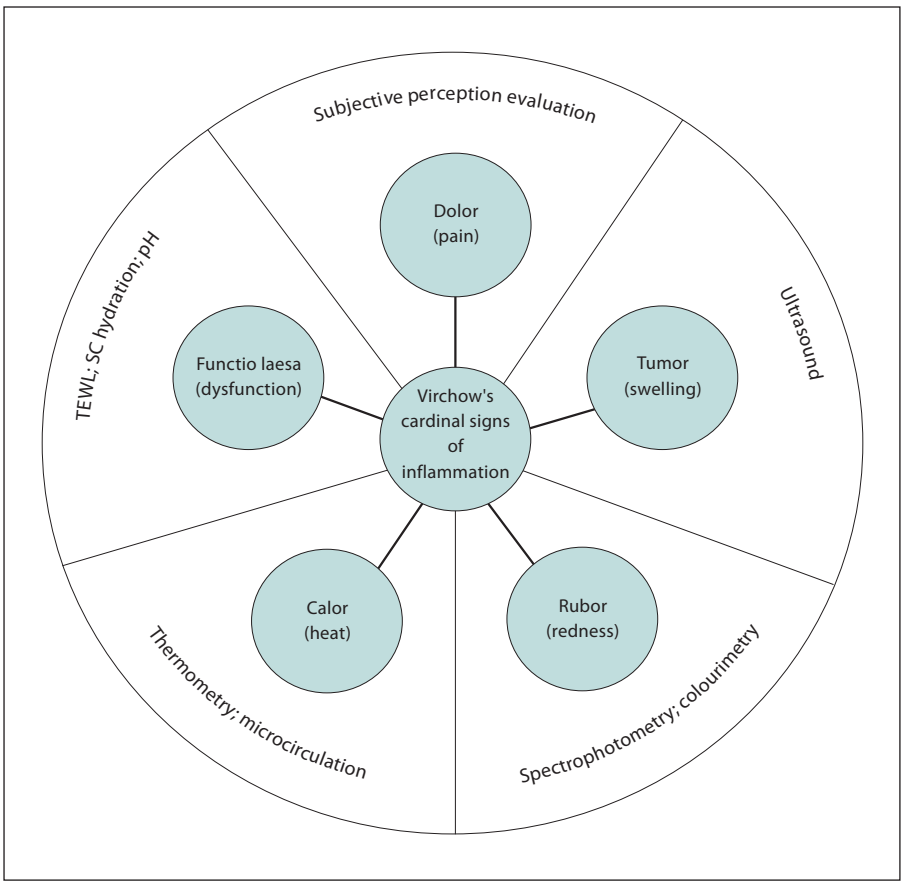

Fig. 2. From Virchow's key signs of inflammation to bioengineering in skin irritation in vivo.

Microcirculation

Several techniques – LDV, laser Doppler imaging (LDI), photoplethysmography, thermal conductance, thermography and xenon wash-out techniques – can be used to investigate blood flow in cutaneous microcirculation. LDV and LDI are the most widely used instruments for the assessment of microcirculation [106]. It was demonstrated that skin blood flow, assessed by LDV, may have to be raised 3–4 times before the naked eye can detect changes [107]. Furthermore, using LDI Fullerton et al. [108] evidenced that the area with increased perfusion exceeded the area with a clinically visible irritant reaction.

In the time course of an irritant reaction, while microcirculation assessed by LDV has already decreased, chromametry continues to register increased a* values [62]. A reasonable explanation of this is the sequential setting of vasodilatation and oedema, thus producing compression of the blood vessels which influences the movement of the erythrocytes in the blood flow.

In general, cutaneous microperfusion increased after exposure to SLS and other model irritants [39, 62, 69]. The peak of the reaction after single closed-chamber exposure of SLS (1%, 2%) was observed on day 2 [39, 69]. However, other data suggest that changes in microcirculation induced by low doses (0.125%) and short term (3 and 6 h) of a single contact could not be registered by LDV [63]. The authors concluded that LDV is useful when SLS concentration is $\geq$0.5% and its occluded single application time is $\geq$6 h [63]. In a long-term repetitive model of irritation, water, acetone and SLS 0.025% never achieved a significant increase in LDV values [105]. Laser Doppler microperfusion evaluation was revealed useful in discrimination in the time course of dithranol-, tretinoin- and UV-induced irritation [39]. These findings support the different pathogenetic mechanisms involved in the varying irritation patterns. As different factors can affect the results, comprehensive guidelines on performing LDV and LDI have been published [106, 109, 110].

Cross-Sectional Methods

Other non-invasive techniques for studying cutaneous irritancy have been introduced in the last decades. A

number of studies apply ultrasound evaluation of the skin reaction elicited by irritant exposure [111–113]. The basis of using this method is the changes of skin thickness due to dermal oedema which were initially registered by A-mode ultrasound scanning [113]. Introducing the two-dimensional B-mode ultrasound technique revealed more intimate changes in the epidermis [111, 112]. It was demonstrated that SLS application induces hypo-echogenicity of the epidermis, while non-anoic and hydrochloric acid elicit an increase in the entrance echo [111]. Furthermore, 20-MHz B-scan recordings were also proven to be efficient in the evaluation of subclinical irritation [112].

Newly developed technologies, such as optical coherence tomography and in vivo confocal reflectance microscopy, are applied in the scope of skin irritation [16, 17]. Structural changes in the living skin exposed to irritants were revealed by application of confocal reflectance microscopy [16, 114]. SC disruption, epidermal necrosis and hyperproliferation were revealed as hallmarks of ICD, while others such as spongiosis, exocytosis, vesicle formation and vasodilatation were also observed in the course of contact hypersensitivity reaction [16].

Different spectroscopic methods were introduced in investigating irritancy on the molecular level [77, 91, 115]. SC barrier properties and transcutaneous penetration of different substances have recently been demonstrated by in vivo Raman microspectrometry [77, 91, 116]. However, one should keep in mind that the newly developed methods are at the dawn and time is needed to standardize the protocols and summarize the cumulated experience.

Many non-invasive techniques for investigating irritation have been reviewed, but there is no single universal one that could be used. Figure 2 presents the use of non-invasive methods for in vivo assessment of the major patterns of irritant-induced inflammation.

In summary, the variety of physiological changes caused by the diverse irritants indicates the need for implementation of a multiparametric approach in the evaluation of irritant reaction [39]. A modern statistical method, i.e. classification tree, was proposed for the assessment of allergic patch reactions by means of different non-invasive techniques [117]. The lack of a subjective component and the good predictive ability raise the idea of a possible application of such a multiparametric and non-invasive approach in screening irritants and irritant reactions in vivo.

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